THIN-LAYER CHROMATOGRAPHIC STUDIES OF SOME WATER POLLUTANTS CONTAINING CARBOXYLIC GROUP

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DECLARATION

I certify that the work presented in the following pages has been carried out by Mr. Sushil Kumar Saxena and that it is suitable for submission towards partial fulfilment of the requirements for the degree of Master of Philosophy in Chemistry of the Aligarh Muslim University, Aligarh.

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C O N T E N T S

LIST OF TABLES

LIST OF PUBLICATIONS

1. "Sequential Thin-Layer Chromatography of 2,4-D and Related Compounds", H.S.Rathore and S.K.Saxena, Journal of Liquid Chromatography, 10(16), 3623-3636 (1987).

ABSTRACT

Separations and identification of pollutants, carboxylic herbicidal substances, such as 2,4-dichlorophenoxyacetic acid, trichloacetic acid, 2,4,5-trichlorophenoxyacetic acid and plant growth regulators such as benzoic acid, cinnamic acid, indole-3-acetic acid, β-naphthaleneacetic acid, β-naphthoxyacetic acid, phenoxyacetic acid have been made by sequential thin-layer chromatography on calcium sulphate layers with acetone, benzene, carbon tetrachloride, chloroform, ethyl acetate, dioxan, propanol as solvents and bromophenol blue as detector.

C H A P T E R - I

GENERAL INTRODUCTION

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Thin-layer chromatography (TLC) is a quick and simple semi-quantitative analytical technique. It requires inexpensive appratus for many routine applications. Though TLC is lacking the precise specificity of gas-liquid chromatography, it is more precise and more sensitive than paper chromatography (PC). It provides much information quickly which would have been difficult or impossible to get in any other way. When TLC coupled with other sophisticated analytical techniques it becomes a powerful tool and often plays a vital role in the separation of complex organic mixtures. There are, however, some limitations to this technique. Resolving power of TLC is limited because of chromatographic conditions e.g. mobile phase velocity is difficult to control.

It is well known that TLC has been, and still is being, used for a wide variety of applications within the organic chemical industry. The use of TLC as a rapid and simple spot test either in the laboratory or in the field, is likely to remain with us for many years to come. Over the last decade, the technique has been widely applied in areas such as lipids, terpenoids and essential oils, vitamins, steroids, alkaloids, pharmaceuticals, drugs, antibiotics, clinical medicine, plant extracts, food additives, pesticides, protiens, amino acids,

nucleotides, nucleic acids, sugars and inorganics. Not only does TLC play a part in the research, development and process and quality control of these materials, it also fulfils vital role in analytical schemes devised to unravel the composition of unknown mixtures. It is also useful in solving the problems concerned with conservation of the environment. These applications have been adequately chronicled in the several books on TLC. For example, books edited by Stahl[1] and Kirchner[2] are very comprehensive.

(i) Recent advances in thin-layer chromatography as a technique

The great majority of work was performed on silica gel layers, followed by chemically bonded reversed-phase (RP) layers, cellulose, layers impregnated with various reagents, ion-exchanger, polyamide and alumina. Interest continues to be high in fundamental studies of the mechanism, theories, and optimization of TLC as well as in some less routine techniques such as over-pressurized, two-dimensional (2-D), multiple development or sequential (S), and ion-pair (IP) TLC. However, most workers continue to employ conventional one-dimensional (P-TLC), ascending development of layers with binary or ternary organic or organic-aqueous mobile phases. TLC on rods with flame ionization detection (FID) was reported in a large number of papers, usually for determinations of lipids or hydrocarbons.

(ii) Significance of less routine techniques

(a) Over-pressurized thin-layer chromatography: As mentioned above that the application of reversed-phase thin-layer chromatography (RP-TLC) on silica gel impregnated with a non-polar stationary phase or silanized or chemically bonded alkylsilyl silica gels[3-8] for separating complex organic mixtures is well known. Ion-pair chromatography on reversed-phase layers (IP-RP-TLC) is also widely used for achieving more selective separations[9]. IP-RP-TLC of quaternary ammonium ions was studied in terms of counteranion concentrations[10] and radii[11] and the alkyl chain length of substituents on the quaternary ammonium cations. The chromatographic properties of 132 compounds were investigated[12] in two ion-pair systems on RP plates, consisting of tetrabutyl and tetramethyl ammonium halides (anionic mode) and sodium heptyl and methylsulfonates (cationic mode). The overall utility of RP-TLC and IP-RP-TLC suffers badly from the incomplete wetness of the layer and from a significant increase in the development time with increasing water content of the developer.

The drawbacks of RP-TLC and IP-RP-TLC have been minimized by over-pressurized thin-layer chromatography (OP-TLC), introduced by Tyihak and Co-workers[13,14] in the year 1979. Recently it has been successfully applied for separating digitalis glycosides[15,16]

and bile acids[17,18]. The main advantages of this technique are (i) it can be used for separating organic mixtures within reasonable development time, and (ii) IP-RP-TLC systems in high-performance liquid chromatography (HPLC) can be modelled by utilizing OP-TLC pre-investigational data.

(b) Two-dimentional thin-layer chromatography: Bi-dimentional chromatography more specific term than two dimentional chromatography when referring to flat paper or thin-layers[19]. In this method the analyte is spotted at the corner of a flat bed and a developer is allowed to migrate in one direction followed by another migration either of the same developer or a new developer at right-angles to the first.

When the same developer is used successively in the two directions of a uniform adsorbent layer, all the spots align on a diagonal. Hence the advantage of the method is very slight increase in resolution corresponding to an increase by a factor of $\sqrt{2}$ in the distance of migration of the spot. The system can be made more efficient, in order to resolve a layer number of spots, by the use of the entire chromatographic area[20] and appropriate different developers for each direction.

Similar analytical potential can also be attained when the same developer is used successively at an orthogonal angle to a chromatographic plate that has been coated with two adsorbents.

The tremendous separation potential of the technique is due to the combined effect of various stationary and mobile phases that can be used in either direction. Zakaria et al.[21] have reviewed the applications of 2-D in separating lipids, pigments, alkaloids, proteins and their constituents, carbohydrates, glycopeptides, nucleic acids and their constituents, environmental pollutants, pesticides, inorganic compounds etc.

(c) Sequential thin-layer chromatography: Sequential thin-layer chromatography (S-TLC) is also known as multiple-development thin-layer chromatography (MD-TLC). In this technique the analyte is spotted as usual and a developer is allowed to ascend a small part of the chromatogram, the plate is removed from the chamber, it is allowed to dry in order to remove the developer and then the second developer is allowed to ascend the distance covered by the first developer as well as some additional fresh part of the chromatogram. Thus a chromatogram can be developed in 2 to 6 different solvents. Similarly a plate can be coated with different adsorbents and it can be developed in the same solvent system. The advantage of the technique is the following: ternary and quaternary separations can be achieved by multiple development of the same plate while only binary separations are possible by single development with any one of the solvents. For example a mixture contains three compounds A, B and C and their R_f values are 0, 1, 1 and 0, 1, 0 in developers d_1 and d_2 respectively.

In single development binary separations such as A from B and C in solvent d_1 and A and C from B in solvent d_2 are possible. In bi-development if chromatogram is developed from 0 to 5 cm with solvent d_1 and after removing d_1 it is developed 0 to 10 cm with solvent d_2 the R_f values will be 0, 1 and 0.5 for compound A, B and C respectively. Hence ternary separation, A-C-B, can be achieved. Some recently reported papers in this area are summarized in the following paragraph.

Abou-Donia and Komeil[22] have used S-TLC for the separation and determination of paraquat dichloride and five related compounds on glass fiber sheets impregnated with silicic acid using benzene-amyl alcohol-methyl alcohol-1N hydrochloric acid (1:1:2:1) then methyl cyanide-water-ammonia (40:9:1). Abou-Donia[23] has also separated leptophos, debromoleptophos, oxon, 4-bromo-2,5-dichlorophenol, o-methylphenylphosphonothioate and phenyl-phosphonic acid on the same material using methyl cyanide-water-ammonia (40:9:1) then n-hexane-ethyl ether (90:10) as the solvents and iodine as the detector. Abou-Donia and Ashry[24] have separated o-ethyl-O-4-nitrophenyl, phenylphosphonothioate and related compounds on the same material using methyl cyanide-water-ammonia (40:9:1) and then hexane-CH-acetic acid (5:5:1). Riebel and Reilich[25] have analysed methamidophos in potato tubers and foliage on silufol plates using hexane-acetone (1:1) followed by chloroform-acetone-methanol (1:1:1) solvent systems and the spots have been located

by spraying a dilute swine liver homogenate and then with alcoholic β-naphthol acetate. Thomas et al.[25] have used gas chromatograph in the sequential configuration analysis of synthetic H-Val-Ala-Leu-Ala-Lys-Lys-Ile-Leu-NH_2. The method involved the treatment of peptide with tert-BuNCO to give the tert-butylcarbamoyl peptide which was cleaved with MeCHOH/HCl to the N-terminal tert-butylcarbamoyl amino acid iso-prester. The latter enantiomeric constituents could be detected by enantioselective gas chromatograph using a 25 mm glass capillary with XE-60-L-valine (δ)-α-phenylethylamide. Nyiredy et al.[27] have separated five bipyridyl isomers by the mobile phase selection was carried out by the use of PRISMA model. The sequential centrifugal layer chromatography (S-C-LC) method was combined with a recycling technique. A review by Bunceak[28] describes S-TLC as an extended TLC to a new fast separation technique that was impossible before. The application of S-TLC are wide spread in all analytical fields of chemistry, medical, biological, and radioactive substances. The solvent supply to the TLC-plate is fully variable in time and space. Analytical time can be reduced to a small part of its usual values. Faupel et al.[29] applied S-TLC to purify radiolabled steroids with (3H) aldosterone. Bunceak[30] has also discussed principles and applications of a new rapid separation method, S-TLC in a review with sixteen references.

(iii) Thin-layer chromatography and analysis of herbicidal pollutants

Plant growth regulators such as benzoic acid, cinnamic acid, indole-3-acetic acid and its derivatives, gallic acid, TCA and 2,4,5-T are difficult to analyse by largely used and highly sensitive technique, "Gas Chromatography" due to their non-volatile nature. Therefore, simple and commonly used methods[31] such as spectrophotometry and TLC have been proved to be the readily available tool for their analysis. Some of the common properties including health hazards of the above compounds are summarized in table 1.

Zawadska et al.[32] have determined 2,4-D, dalapon, MCPA, dichlorprop, mecoprof, DNOC, dinoseb and TCA in water and sewage by TLC on silica gel G-Kieselguhr G (2:3) or silica gel G-H_3PO_4 (5 g of silica gel G in 17 ml of 0.3% H_3PO_4 solution) layers with light petroleum-isopropyl ether (1:2) as solvent. Ethanolic diphenylamine have been used as a location reagent. Thielmann[33] has separated and identified carboxylic herbicidal substances on UV 254 sheets with benzene-acetone (2:3) or (4:2) as the solvent and 0.02% ethanolic Rohdamine B as the spray reagent. Bogacka and Taylor[34] have determined these pesticides in water by TLC on silica gel G-Kieselguhr G (2:3) with paraffin as solvent and

0.5% $AgNO_3$ solution as spray reagent. Ahmed et al.[35] have claimed that calcium sulphate is an excellent TLC material. They have separated plant growth regulator such as benzoic, cinnamic, indole-3-acetic, β-naphthaleneacetic, β-naphthoxyacetic and phenoxyacetic acids from many carboxylic acids present in plants, fruits and soils.

In this dissertation an attempt has been made to study the separation potential of S-TLC on calcium sulphate in common solvents for 18 herbicidal pollutants.

TABLE 1

SOME CARBOXYLIC POLLUTANTS AND THEIR ACTIONS AND HAZARDOUS EFFECTS.

Carboxylic pollutants	LD_{50} mg/kg (rat) oral	Actions	Hazardous effects
Benzoic acid	-	Plant growth regulator (inhibitor of plant physiological-processes).	Mild irritant to skin, eyes and mucous membrane, its vapour causes coughing and sneezing.
Cinnamic acid	-	Plant growth regulator.	Mild local irritant and slightly toxic.
2,4-D/2,4-dichlorophenoxyacetic acid	375	Herbicide, postmergent control of weeds.	Very toxic, carcinogenic and irritant to eyes.
Gallic acid/3,4,5-trihydroxy benzoic acid	-	Plant growth regulator.	Mild local irritant and slightly toxic.
IAA/Indole-3-acetic acid	-	Plant growth regulator.	Irritant.
β -naphthaleneacetic acid	1000	Plant growth regulator.	Mild irritant.
β -naphthoxyacetic acid	-	Blossom set and growth regulator.	Mild irritant.
Phenoxyacetic acid	-	Herbicide.	Mild irritant.
TCA/Trichloroacetic acid	5000	Control of weed grass on non-crop land, control seedling grasses and some broad leaf weeds in sugar beet and sugar cane.	Toxic, irritant to skin, eyes and gastric irritation on inhalation.
2,4,5-T/2,4,5-trichlorophenoxyacetic acid	300	Herbicide (specially for bush control)	Very toxic and irritant to eyes and skin.

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C H A P T E R - II

SEQUENTIAL THIN-LAYER CHROMATOGRAPHY OF 2,4-D AND RELATED COMPOUNDS

INTRODUCTION

Our previous work[1] shows that benzoic acid, cinnamic acid, indole-3-acetic acid and its derivatives, gallic acid, TCA and 2,4,5-T can be separated by P-TLC on calcium sulphate coating in common solvents such as acetone, benzene, carbon tetrachloride, chloroform, dioxan, ethyl acetate and propanol. Abou-Donia and Komiel[2] have claimed that the newly developed S-TLC is a rapid and fast method of analysis of complex mixtures. Therefore, an attempt has been made to explore the utility of calcium sulphate for the separation, detection and determination of pollutants, carboxylic herbicidal substances, by S-TLC.

As a result many new ternary separations which were not possible by P-TLC on calcium sulphate have now been achieved. The details of the procedure and the results obtained are discussed in this chapter.

EXPERIMENTAL

Apparatus

A Stahl apparatus with a universale applicator (adjustable thickness of the applied layer from(0.25-2.00 mm), hot air electric drier, glass plates (20 x 4 cm), glass jars (25 x 5 cm), temperature controlled electric oven, centrifugation machine and spectrophotometer were used.

Chemicals

Acetone (BDH,India), benzene (S.M.Chemicals,India), calcium sulphate precipitated powder, carbon tetrachloride, chloroform, dioxan, ethyl acetate, propanol (E.Merck,India), benzoic acid, p-chlorophenoxyacetic acid, cinnamic acid, citric acid, 2,4-dichlorophenoxyacetic acid, gallic acid, indole-3-acetic acid, maleic acid, malic acid, malonic acid, β-naphthaleneacetic acid, β-naphthoxyacetic acid, oxalic acid, phenoxyacetic acid, salicylic acid, tartaric acid, trichloroacetic acid, 2,4,5-trichlorophenoxyacetic acid (Sigma,USA) were used.

Preparation of solutions

Solutions (2%) of benzoic, p-chlorophenoxyacetic, cinnamic, 2,4-dichlorophenoxyacetic, gallic, indole-3-acetic, β-naphthaleneacetic, β-naphthoxyacetic, phenoxyacetic, salicylic and

2,4,5-trichlorophenoxyacetic acids were prepared in ethanol. Solutions (2%) of citric, maleic, malic, malonic, oxalic, tartaric and trichloroacetic acids were prepared in distilled water.

Reagent for the spectrophotometric determination of indole-3-acetic acid was prepared by mixing 1 ml of 0.5M ferric chloride solution in 50 ml of 35% (v/v) perchloric acid. Prepared fresh reagent before use.

Preparation of plates

A slurry of calcium sulphate obtained by mixing calcium sulphate (30 g) with distilled water (70 ml) was applied on the glass plates with the help of applicator so that the thickness of calcium sulphate slurry would be 0.75 mm. The plates were first allowed to dry at room temperature and then in a temperature controlled electric oven at 110°C for one hour.

Detection of acids

The acids under study were located on the plates by 1% ethanolic, alkaline bromophenol blue solution.

Spotting of test solutions

Test solutions were spotted on the plates with the help of a fine capillary. The solvent was removed by hot air drying,

the plate was developed up to the length of 5 cm in solvent A, the plate was taken out from the jar and A was removed as above, the plate was again developed upto the length of 10 cm in solvent B, B was also removed and then the spot was located with bromophenol blue by spray method. For tailing, the front limit (RI) and the rear limit (RT) were measured while for compact spot R_f values were teken as usual.

$$R_f = \frac{\text{Distance travelled by substance (cm)}}{\text{Distance travelled by solvent front (cm)}}$$

In case two spots were observed, the distance of their centres from the point of application have been recorded e.g. 0, 10 means one spot at 0 and second spot at 10 cm.

Qualitative separations

To achieve the qualitative separation of acids, one of the acids was spotted firstly and the solvent was removed, second acid solution was spotted and the solvent was removed and so on so far and then the plates was developed, dried and acids were located as above.

Quantitative separations

A knew volume of standard acid solution was spotted on the plate with the help of a graduated micro pipette with vaccupet

control, the solvent was removed, plates were developed and spots were located as above. The demarcated area of the plate was scratched out, the acid was extracted with methanol (3 ml) and the solid was removed by centrifugation method. The acid in the centrifugate was determined spectrophotometrically by the following procedure.

In the extract (3 ml) containing indole-3-acetic acid, 2 ml of freshly prepared reagent were added dropwise but rapidly with continuous agitation, it was placed in dark for one hour for colour development. Finally the absorbance was measured at 510 nm against a blank containing methanol (3 ml) and reagent (2 ml).

RESULTS

R_f values of compounds under study on calcium sulphate coating by P-TLC are given in table 2. R_f values by S-TLC are given in tables 3, 4 and 5. R_f values calculated by the following formulae and R_f values obtained by S-TLC practically are recorded in table 6.

(i) If the compound has R_f values 0.0 and 1.0 in the solvents a and b respectively by P-TLC.

$$R_f\ ab = \frac{R_f\ a}{2} + R_f\ b \quad \ldots \quad (1)$$

$$R_f\ ba = R_f\ a + \frac{R_f\ b}{2} \quad \ldots \quad (2)$$

(ii) If the compound has R_f values 0.0 and more than zero but less than one in solvents a and b respectively by P-TLC.

$$R_f\ ab = 0.0 \times R_f\ a + R_f\ b \quad \ldots \quad (3)$$

$$R_f\ ba = \frac{R_f\ a + R_f\ b}{2} \quad \ldots \quad (4)$$

(iii) If compound is tailing by P-TLC.

$$R_f\ ab = 0.0 \times R_f\ a \text{ (mid of tailing)} + R_f\ b \quad (5)$$

$$R_f\ ba = \frac{R_f\ a + R_f\ b \text{ (mid of tailing)}}{2} \quad (6)$$

Where R_f a and R_f b are R_f values in the solvents a and b respectively by P-TLC. R_f ab and R_f ba are the R_f values by S-TLC when solvent a followed by solvent b and solvent b following by solvent a respectively.

Separations achieved by P-TLC are recorded in table 7. Some of the important separations achieved by S-TLC are shown in photoplates 1 to 8 and others are given in tables 8 and 9. The results of quantitative separations are given in tables 10, 11 and 12. The analytical parameters are calculated by following expressions (7,8).

$$\sigma = \sqrt{\frac{(x_1-\mu)^2 + (x_2-\mu)^2 + \ldots.}{N-1}} \qquad \ldots \qquad (7)$$

$$C.V. = \frac{\sigma \times 100}{\mu} \qquad \ldots \qquad (8)$$

where x_1, x_2 .... = measured values

μ = average value, and

N = number of sets.

TABLE 2

R_f VALUES OF SOME CARBOXYLIC POLLUTANTS ON CALCIUM SULPHATE COATING BY P-TLC.

Carboxylic pollutants	a	b	c	d	e	f	g
BA	1.0	0-6	0-9	0-6	1.0	1.0	1.0
CIA	1.0	1.0	0-10	3-10	1.0	1.0	1.0
CA	0-4	0.0	0.0	0.0	0-3	0-9	1.0
CPX	1.0	0.0	0.0	0-6	8-10	1.0	1.0
2,4-D	1.0	0-4	0.2	0-7	1.0	1.0	1.0
GA	1.0	0-4	0.0	0.0	7-10	1.0	1.0
IAA	1.0	8-10	0-5	0-5	1.0	1.0	1.0
MEA	0,10	0-3	0.0	0.0	0-10	0-10	0-10
MA	0,10	0-8	0-6	0-6	0-10	0-10	1.0
NOA	0,10	0.0	0.0	0.0	1.0	0-10	0-10
NPA	1.0	4-9	0-10	0-10	1.0	1.0	1.0
NPX	1.0	0-7	0-5	0-5	1.0	1.0	1.0
OA	5-10	0.0	0.0	0.0	0.0	0-5	0-10
PHX	1.0	8-10	0-8	0-8	1.0	1.0	1.0
SA	1.0	8-10	4-10	4-10	1.0	1.0	1.0
TA	0-9	0-5	0.0	0.0	0.0	0-5	0-7
TCA	1.0	0.7	0.5	0-5	1.0	1.0	1.0
2,4,5-T	1.0	0.6	0.3	0-7	1.0	1.0	1.0

Abbreviations used are:

BA - benzoic acid; CIA - cinnamic acid; CA - citric acid; CPX - p-chlorophenoxyacetic acid; 2,4-D - 2,4-dichlorophenoxyacetic acid; GA - gallic acid; IAA - indole-3-acetic acid; MEA - maleic acid; MA - malic acid; MOA - malonic acid; NPA - β-naphthalene-acetic acid; NPX - β-naphthoxyacetic acid; OA - oxalic acid; PHX - phenoxyacetic acid; SA - salicylic acid; TA - tartaric acid; TCA - trichloroacetic acid; 2,4,5-T - 2,4,5-trichlorophenoxyacetic acid.

a - acetone; b - benzene; c - carbon tetrachloride; d - chloroform; e - ethyl acetate; f - dioxan; g - propanol.

ab - a followed by b; ba - b followed by a; and so on so forth.

TABLE 3

R_f VALUES OF SOME CARBOXYLIC POLLUTANTS ON CALCIUM SULPHATE COATING BY S-TLC.

Carboxylic pollutants	ab	ba	ac	ca	ad	da	ae	ea	af	fa	ag	ga	be	eb
BA	6-7	1.0	5-8	1.0	5-8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0-9	0-6
CIA	1.0	1.0	5-10	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	5-7	1.0
CA	0-5	0-5	0-5	6-10	0.5	0-4	0-10	0-10	0-9	0-9	0-10	0-10	0.0	0.0
CPX	0.5	1.0	0.5	1.0	5-8	1.0	5-8	1.0	1.0	1.0	1.0	1.0	0.0	0.0
2,4-D	0.6	1.0	0.6	1.0	6-8	1.0	5-6	1.0	1.0	1.0	1.0	1.0	5-7	2-5
GA	5-7	1.0	0.5	1.0	0.5	1.0	0.5	1.0	1.0	1.0	1.0	1.0	0.4	0-4
IAA	5-10	1.0	5-7	7-10	5-10	1.0	9-10	9-10	1.0	1.0	8-10	9-10	0-2	1.0
MEA	0.5	0,10	0-5	0,10	0,5	0-10	0-10	0-10	0-10	0-10	0-10	0-10	0.0	0.0
MA	0-10	0,10	0-7	0,10	0,10	0-10	0-10	0-10	0-10	0-10	0-10	0-10	0-5	0-10
MOA	0.5	7-10	0-5	0,10	0-5	0-10	0-10	1.0	0-10	0-10	0-10	0-10	0-0	0-0
NPA	5-10	1.0	5-8	1.0	8-10	1.0	1.0	1.0	1.0	1.0	1.0	1.0	2-7	6-10
NPX	6-7	1.0	0.5	1.0	5-8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0-3	3-6
OA	0.3	0.0	0-5	0.0	0-5	0.0	0-5	0-5	0-5,10	0-10	0-5	0-5	0.0	0.0
PHX	0.9	1.0	5-9	1.0	5-10	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0-7	0-7
SA	5-10	1.0	4-8	1.0	5-10	1.0	1.0	1.0	1.0	1.0	1.0	1.0	2-7	0.5
TA	0-4	0.0	1-2	0.0	0-2	0.0	0-6	0-6	0-9	0-9	0-5	0-4	0.0	0.0
TCA	5-6	1.0	0.7	1.0	5-7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	5-7	5-7
2,4,5-T	0.8	1.0	0.6	1.0	5-8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	6-8	0-5

Abbreviations are defined in table 2.

TABLE 4

R_f VALUES OF SOME CARBOXYLIC POLLUTANTS ON CALCIUM SULPHATE COATING BY S-TLC.

Carboxylic pollutants	bd	db	be	eb	bf	fb	bg	gb	ed	de	ce	ec	ef	fe
BA	0-5	0-4	1.0	5-7	1.0	5-10	1.0	0-7	0-5	0-7	1.0	0-6	1.0	5-10
CIA	5-10	1.0	1.0	1.0	1.0	5-10	1.0	8-10	1.0	4-10	1.0	1.0	1.0	5-10
CA	0.0	0.0	0-5	0-6	0-9	0-5	0-7	0-5	0.0	0.0	0.5	0.5	0-9	0-5
CPX	0-6	0-5	1.0	4-5	1.0	0.5	1.0	8.7	0-5	0.6	8-10	0.4	1.0	0.5
2,4-D	0-6	0-2	1.0	5-6	5-10	5-7	1.0	0.6	1-5	0.6	1.0	0.6	1.0	5-7
GA	0.4	0-5	7-8	5-6	1.0	0-5	1.0	0.6	0.0	0.0	7-9	3-5	1.0	0-5
IAA	0-7	5-8	5-7	1.0	1.0	5-7	1.0	5-7	1.0	0-5	1.0	0.5	1.0	0-5
MEA	0.0	0.0	0.5	1.0	0-10	0.5	0-10	0.5	0.0	0-5	0-10	0.5	0-10	0-5
MA	0-9	0-10	5-5	8-10	0-10	0-10	0-10	0-10	0,10	0-6	0,10	0-10	0-10	0-10
MOA	0.0	0.0	0-5	1.0	0-10	0-5	0-10	0.5	0.0	0.0	0,10	0-10	0-10	0-5
NPA	5-10	5-10	5-10	1.0	1.0	5-7	1.0	8-10	8-10	3-7	1.0	5-10	1.0	5-10
NPX	5-7	4-7	5-10	1.0	1.0	5-7	1.0	5-7	7-10	0-5	1.0	0.5	1.0	5-7
OA	0.0	0.0	0-5	0.0	0-10	0-5	0-4	0-5	0.0	0.0	0.0	0-2	0-10	0-5
PHX	5-8	5-10	1.0	1.0	1.0	4-10	1.0	1.0	1.0	0-5	1.0	5-10	1.0	1.0
SA	5-10	5-10	1.0	1.0	1.0	5-7	1.0	1.0	1.0	4-8	1.0	5-10	1.0	1.0
TA	0.0	0.0	0-3	0-4	0-9	0.5	0-6	0-5	0.0	0.00	0.0	0.2	0-5	0-3
TCA	0-3	0-5	1.0	0.7	1.0	0-7	1.0	5-7	3-4	0.4	1.0	0.6	1.0	1.0
2,4,5-T	0.5	0-5	8-10	1.0	1.0	5-9	1.0	1.0	0-6	0-3	1.0	5-6	1.0	1.0

Abbreviations are defined in table 2.

TABLE 5

R_f VALUES OF SOME CARBOXYLIC POLLUTANTS ON CALCIUM SULPHATE COATING BY S-TLC.

Carboxylic pollutants	eg	ge	de	ed	df	fd	dg	gd	ef	fe	eg	ge	fg	gf
BA	0-10	1.0	1.0	5-8	1.0	5-10	1.0	5.7	1.0	1.0	1.0	1.0	1.0	1.0
CIA	1.0	5-10	1.0	1.0	1.0	5-10	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
CA	1.0	0.5	0-5	0-5	0-10	0-5	1.0	0.5	0.8	0.8	1.0	2-10	0-10	0-9
CPX	1.0	0.5	8-10	4-6	1.0	5-10	1.0	0.5	1.0	1.0	1.0	8-10	1.0	1.0
2,4-D	1.0	0.5	1.0	5-6	1.0	5-7	1.0	5-7	1.0	1.0	1.0	1.0	1.0	1.0
GA	1.0	0.5	7-10	4-5	1.0	0-5	1.0	5-8	0.9	1.0	1.0	7-10	7-10	1.0
IAA	1.0	0.5	1.0	5-10	1.0	5-10	1.0	5-10	1.0	1.0	1.0	8-10	1.0	1.0
MEA	1.0	0.5	1.0	3-5	0-10	0-6	0-10	0-5	0-10	0-10	1.0	1.0	0-10	0.10
MA	1.0	0.5	1.0	2-7	0-10	0-10	0-10	5-10	0-10	0-10	1.0	7-10	0-10	0,10
MOA	1.0	0.5	1.0	0-5	0-10	0-5	0-10	0-5	0-10	0-10	1.0	1.0	0-10	0.10
NPA	1.0	5-10	1.0	8-10	1.0	5-10	1.0	1.0	5-10	1.0	1.0	1.0	1.0	1.0
NPX	1.0	0.5	1.0	7-10	1.0	5-10	1.0	8.10	1.0	1.0	0-10	0-10	1.0	1.0
OA	0.0	0.0	0.0	0.0	0-5	0-2	0-10	0-5	0-6	0-6	1.0	1.0	0-10	0-9
PHX	1.0	1.0	1.0	5-10	1.0	5-10	1.0	8-10	1.0	1.0	1.0	1.0	1.0	1.0
SA	1.0	5-10	1.0	5-10	1.0	5-10	1.0	5-10	1.0	1.0	1.0	1.0	1.0	1.0
TCA	1.0	0.6	1.0	0.6	1.0	5-7	1.0	5-6	1.0	1.0	1.0	1.0	1.0	1.0
2,4,5-T	0.9	0.6	0.9	0.7	1.0	5-7	1.0	5-7	1.0	1.0	1.0	1.0	1.0	1.0

Abbreviations are defined in table 2.

TABLE 6

COMPARISON OF THEORITICAL AND PRACTICAL R_f VALUES BY S-TLC.

Carboxylic pollutants	Solvents - R_f values															
	T	P	T	P	T	P	T	P	T	P	T	P	T	P	T	P
CA	bg		gb		cg		ge		dg		gd		-		-	
	1.0	0-7	0.5	0-5	1.0	1.0	0.5	0.5	1.0	1.0	0.5	0.5				
CPX	ab		ba		ac		ca		bg		gb		cg		ge	
	0.5	0.5	1.0	1.0	0.5	0.5	1.0	1.0	1.0	1.0	0.5	0.7	1.0	1.0	0.5	0.5
2,4-D	ac		ca		ce		ea		cf		fa		cg		ge	
	0.7	0.6	1.0	1.0	0.6	0.6	1.0	1.0	0.6	5-7	1.0	1.0	0.6	0.5	1.0	1.0
GA	ac		ca		ad		da		df		fd		dg		gd	
	0.5	0.5	1.0	1.0	0.5	0.5	1.0	1.0	1.0	1.0	0.5	0-5	0.5	5-8	1.0	1.0
MOA	be		eb		ee		ee		de		ed		-		-	
	0.5	0-5	1.0	1.0	1.0	0,10	0.5	0-10	1.0	1.0	0.5	0-5				
TCA	ac		ca		cf		fe		ef		fe		eg		ge	
	1.0	1.0	0.75	0.7	1.0	1.0	0.75	1.0	1.0	1.0	0.75	1.0	1.0	1.0	0.75	0.6
2,4,5-T	ab		ba		be		eb		bf		fb		bg		gb	
	0.8	0.8	1.0	1.0	0.8	8-10	1.0	1.0	1.0	1.0	0.8	1.0	0.8	1.0	1.0	1.0

T = Theoretical, P = Practical.

TABLE 7

BINARY SEPARATIONS OF SOME CARBOXYLIC POLLUTANTS BY P-TLC

Herbicide	Separated from	Solvents
BA (1.0)	CA (0.0), OA (0.0) and TA (0-2)	a and e
CIA (1.0)	CA (0.0), MBA (0-3), MA (0-7), MOA (0.0), OA (0.0) and TA (0-2)	a, b and e
CPX (1.0)	CA (0.0), OA (0.0) and TA (0-2)	a and e
2,4-D (1.0)	CA (0.0), OA (0.0) and TA (0-2)	a and e
GA (1.0)	CA (0.0), OA (0.0) and TA (0-2)	a and e
IAA (1.0)	CA (0.0), MBA (0-3), MBA (0.0), OA (0.0) and TA (0-2)	a, b and e
MOA (1.0)	CA (0.0), OA (0.0) and TA (0-2)	e
NPA (1.0)	CA (0.0), MBA (0.3), MOA (0.0), OA (0.0) and TA (0-2)	d and e
NPX (8-10)	CA (0.0), OA (0.0) and TA (0-2)	a, c and f
PHX (1.0)	CA (0.0), MBA (0-3), MA (0-7), OA (0.0) and TA (0-2)	a, b and d
SA (1.0)	CA (0.0), MOA (0.0), OA (0.0) and TA (0-2)	c and e
TCA (1.0)	CA (0.0), OA (0.0) and TA (0-2)	a and e
2,4,5-T (1.0)	CA (0.0), OA (0.0) and TA (0-2)	a, b and e

Abbreviations are defined in Table 2.

TABLE 3

BINARY SEPARATIONS OF SOME CARBOXYLIC POLLUTANTS BY S-TLC.

Herbicide	Separated from	Solvents
CIA (1.0)	IAA (0-5), MEA (0-3) and MOA (0.0)	cd, ed and db
IAA (6-10)	CA (0-2), MEA (0-3) and MOA (0.0)	db and gb
MEA (8-10)	CA (0-2), OA (0.0) and TA (0.0)	de and fe
MA (7-10)	CA (0-2), OA (0.0) and TA (0.0)	de and fe
MOA (7-10)	CA (0-2), OA (0.0) and TA (0-2)	de and fe
NPA (6-10)	MEA (0-3) and MOA (0.0)	db
NPX (5-8)	MA (0-3) and (MOA (0.0)	db
PHX (8-10)	IAA (0-5), MEA (0-3), MA (0-5) and MOA (0.0)	db, gb and ge
SA (1.0)	MEA (0-3) and MOA (8-10)	db and gb
2,4,5-T (8-10)	MEA (0-3)	gb

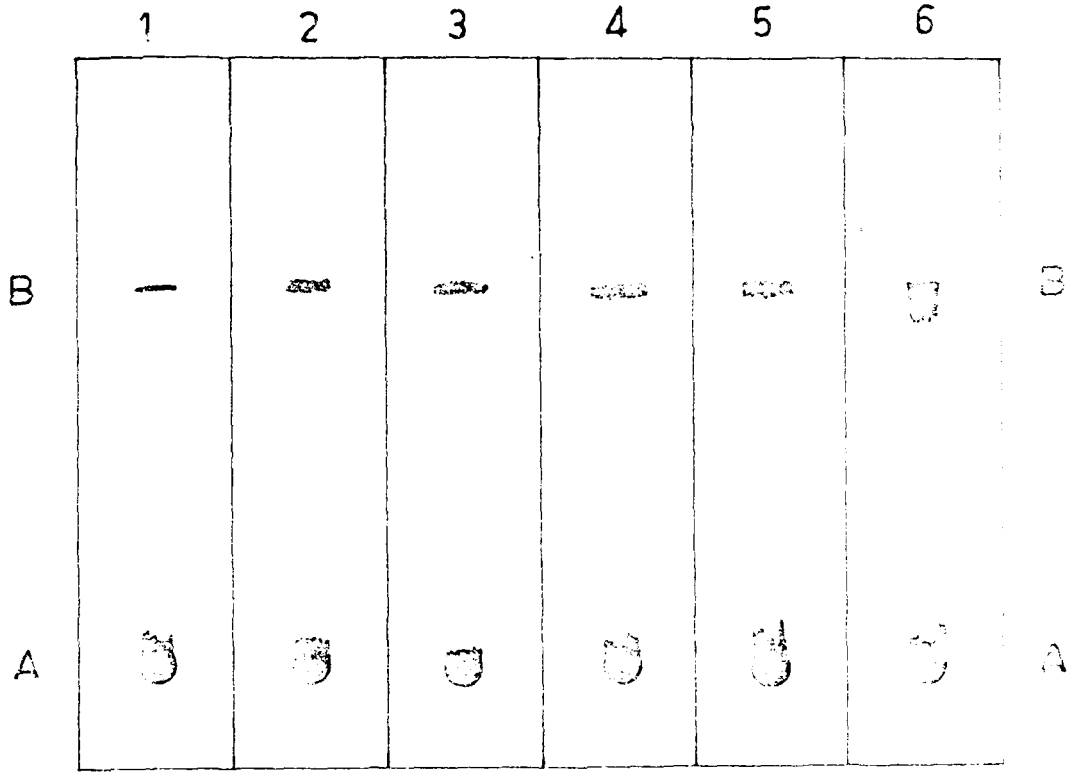
Abbreviations are defined in table 2.

TABLE 9

TERNARY SEPARATIONS OF SOME CARBOXYLIC POLLUTANTS BY S-TLC.

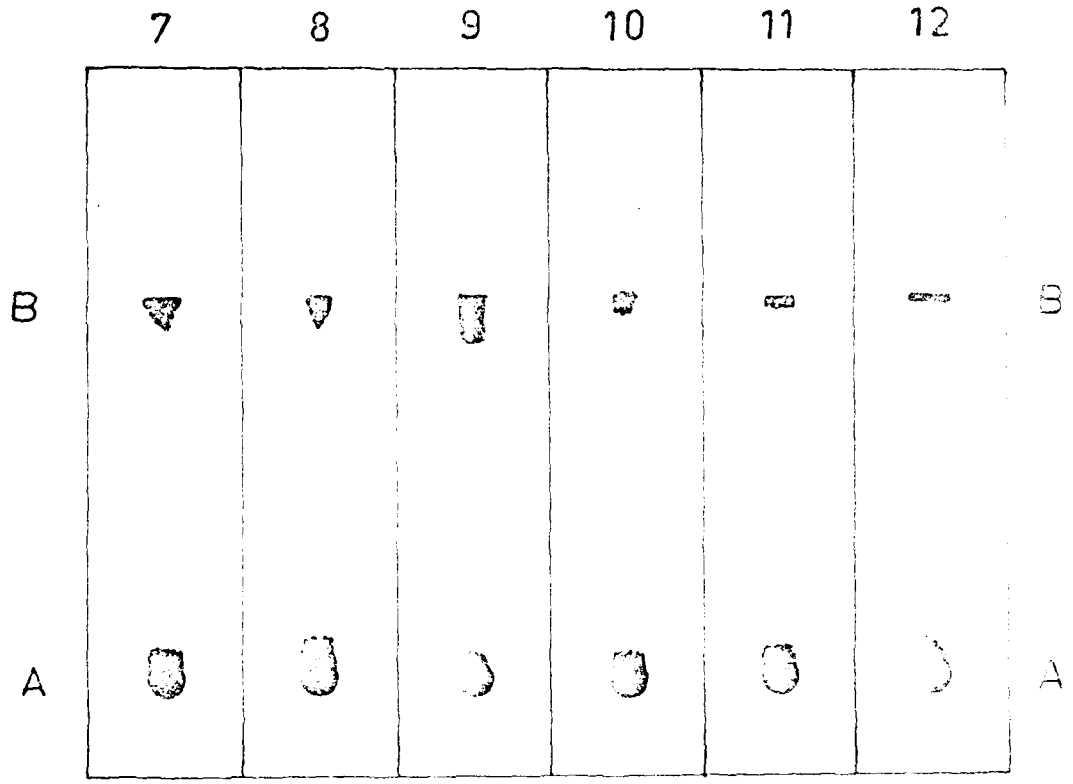
Herbicide	Separated from			Solvent
OA (0.0)	MEA (0.5)	-	NPA (1.0)	ed
OA (0.0)	MEA (4-5)	-	NPX (9-10)	gd
TA (0.0)	NPX (0.5)	-	CIA (8-10)	eb
TA (0.0)	IAA (3-5)	-	SA (9-10)	ab
TA (0.0)	MA (0.5)	-	SA (9-10)	ee
TA (0.0)	MA (0.5)	-	CIA (9-10)	de
TA (0.0)	IAA (4-5)	-	CIA (1.0)	ee
TA (0.0)	IAA (4-5)	-	PHX (9-10)	ae
OA (0.0)	MA (0.5)	-	CIA (9-10)	de
OA (0.0)	IAA (3-5)	-	PHX (1.0)	ee
OA (0.0)	MFA (0.5)	-	PHX (1.0)	ab

Abbreviations are defined in table 2.



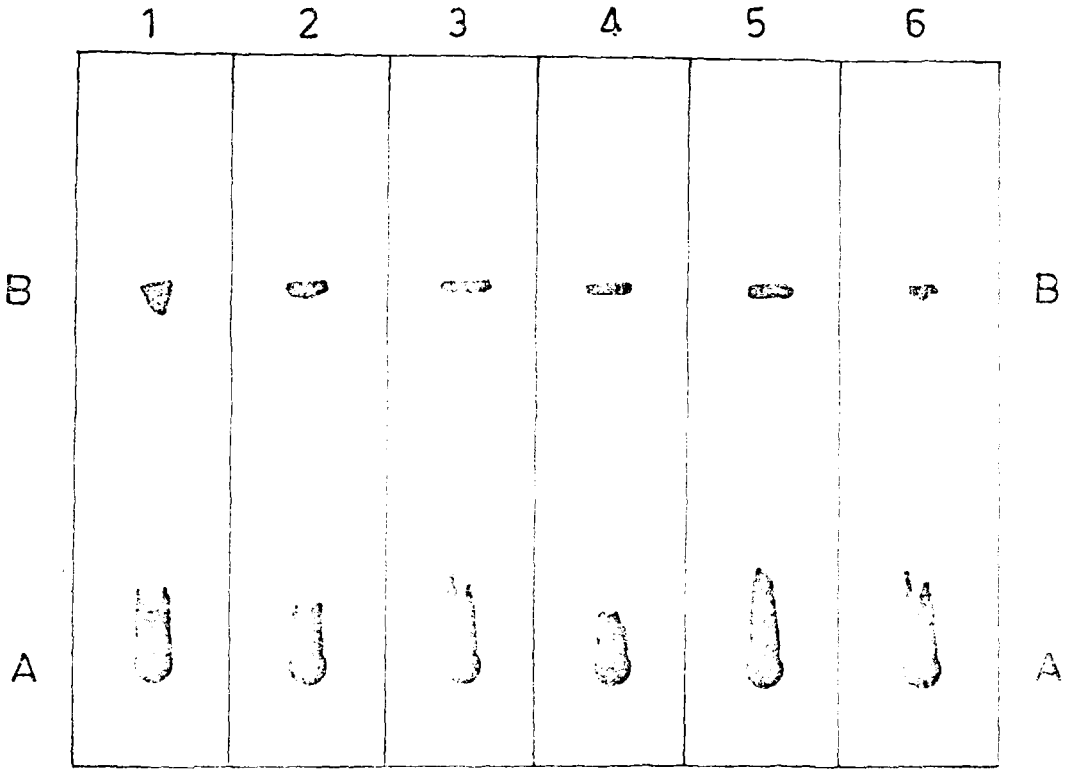
Photoplate No. 1

Separation of tartaric acid (Spot A) from 1) 2,4,5-trichlorophenoxyacetic acid; from 2) 2,4-dichlorophenoxyacetic acid; from 3) benzoic acid; from 4) trichloroacetic acid; from 5) p-chlorophenoxyacetic; acid and from 6) gallic acid; (Spot B) on calcium sulphate coating developed in dioxan (5 cm) followed by ethyl acetate (10 cm).



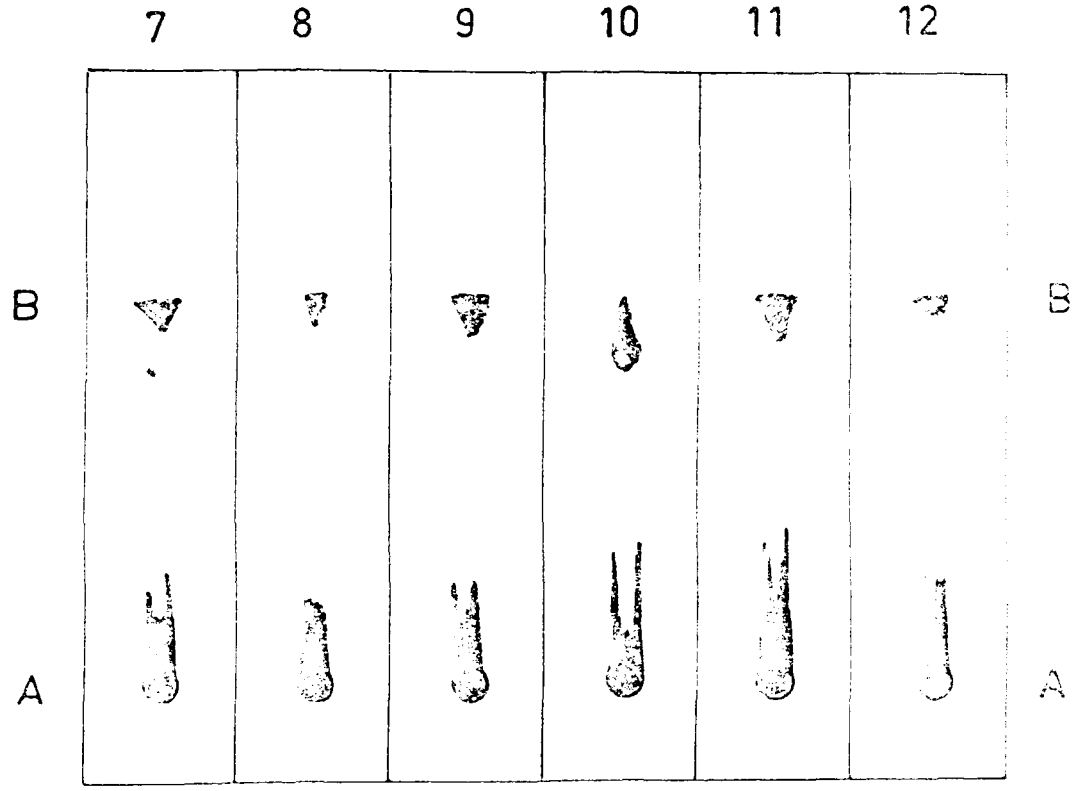
Photoplate No. 2

Separation of tartaric acid (Spot A) from 7) cinnamic acid; from 8) indole-3-acetic acid; from 9) β-naphthaleneacetic acid; from 10) β-naphthoxyacetic acid; from 11) phenoxyacetic acid and from 12) salicylic acid (Spot B) on calcium sulphate coating, developed in dioxan (5cm) followed by ethyl acetate (10 cm).



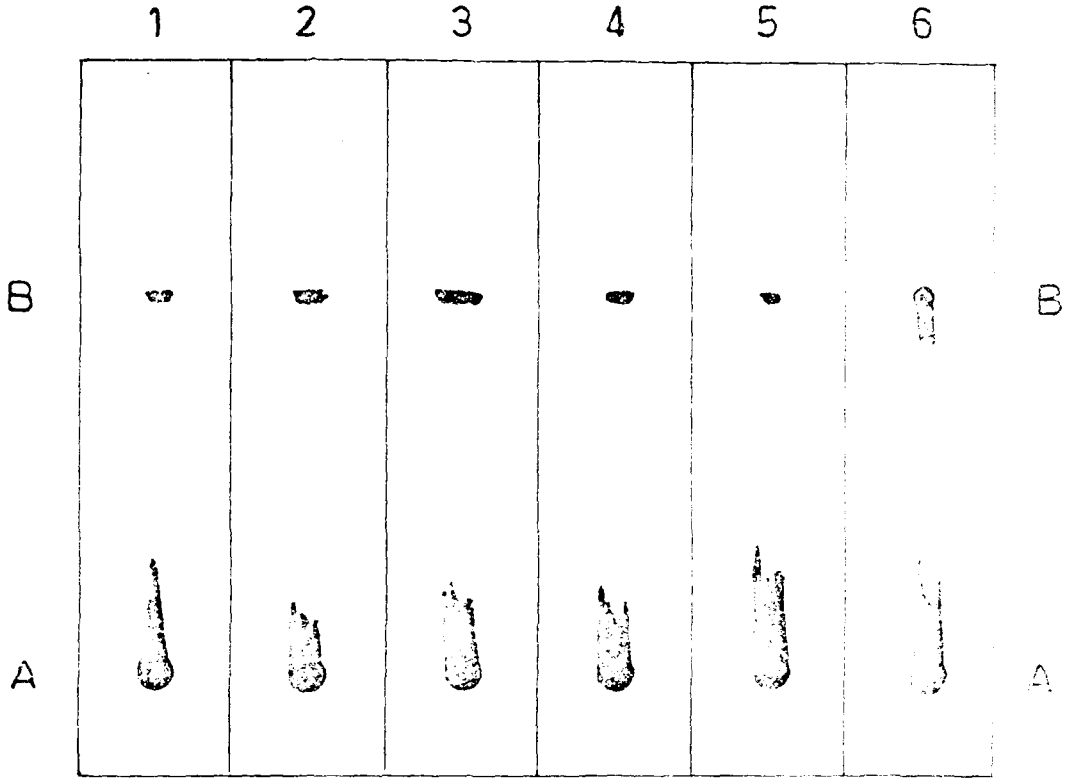
Photoplate No.3

Separation of oxalic acid (Spot A) from 1) 2,4,5-trichlorophenoxyacetic acid; from 2) 2,4-dichlorophenoxyacetic acid; from 3) benzoic acid; from 4) trichloroacetic acid; from 5) p-chlorophenoxyacetic acid and from 6) gallic acid (Spot B) on calcium sulphate coating, developed in dioxan (5 cm) followed by ethy acetate (10 cm).



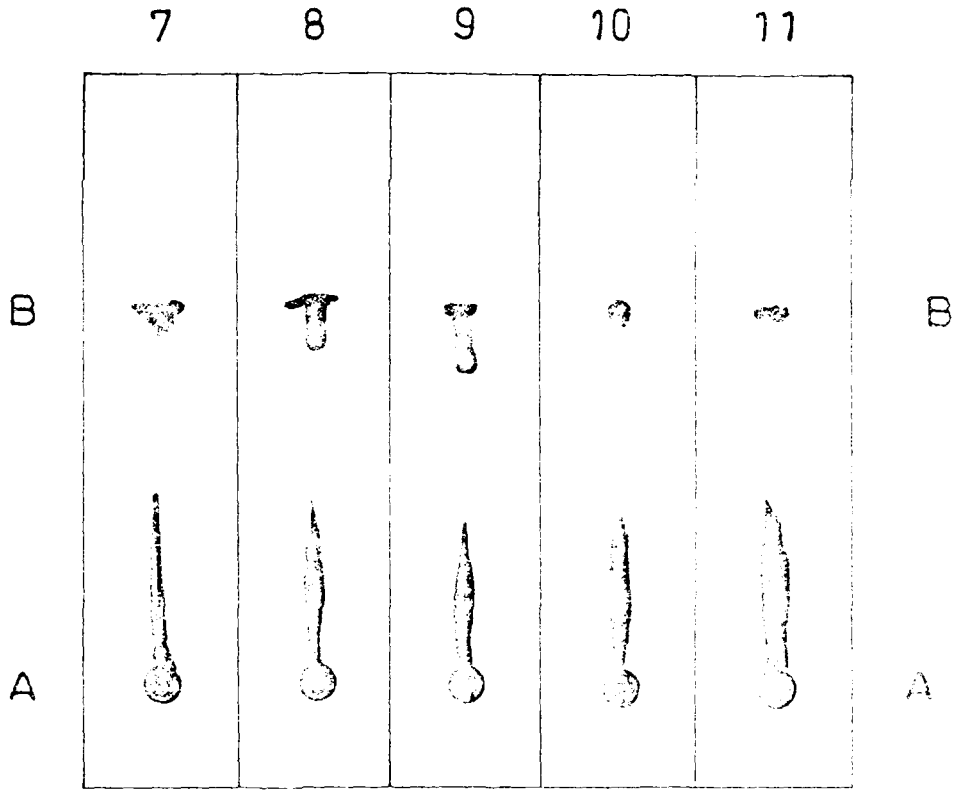
Photoplate No·4

Separation of oxalic acid (Spot A) from 7) cinnamic acid; from 8) indole-3-acetic acid; from 9) β-naphthaleneacetic acid; from 10) β-naphthoxyacetic acid, from 11) phenoxyacetic acid; and from 12) salicylic acid, (Spot B) on calcium sulphate coating, developed in dioxan(5 cm) followed by ethylacetate (10 cm).



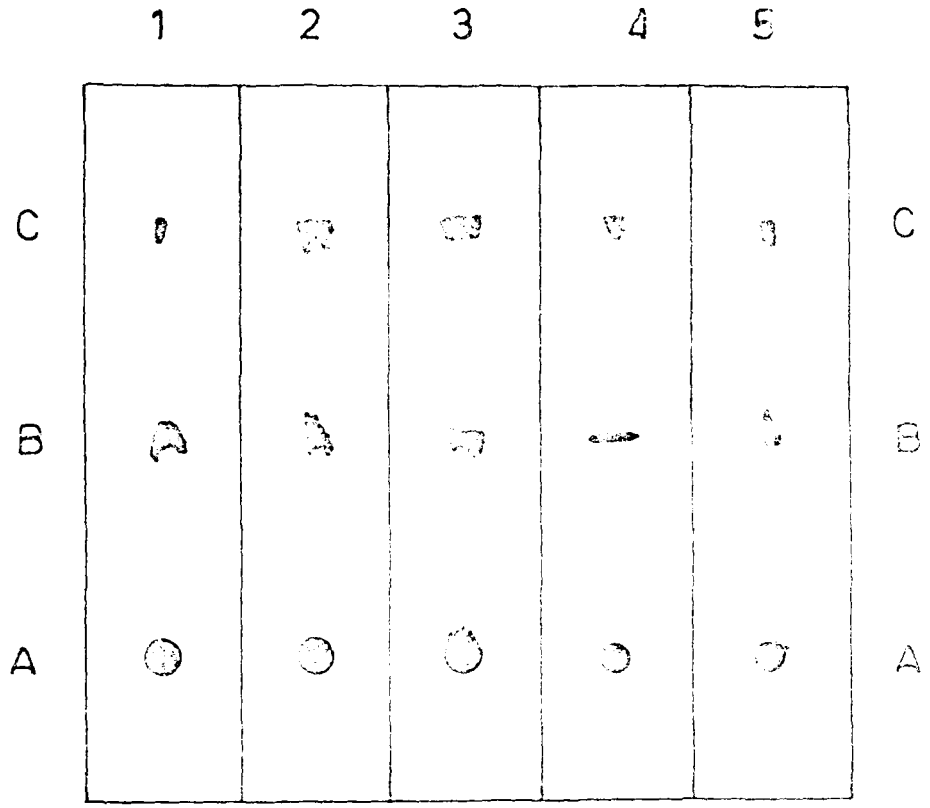
Photoplate No.5

Separation of citric acid (Spot A) from 1) 2,4,5-trichlorophenoxyacetic acid; from 2) 2,4-dichlorophenoxyacetic acid; from 3) benzoic acid, from 4) trichloroacetic acid from 5) p-chlorophenoxyacetic acid and from 6) gallic acid (Spot B) on calcium sulphate coating, developed in dioxan (5 cm) followed by ethyl acetate (10 cm).



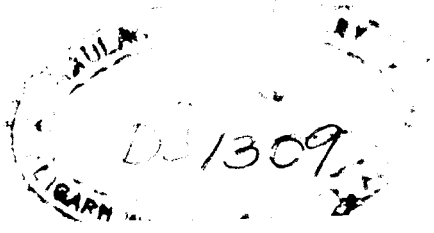
Photoplate No. 6

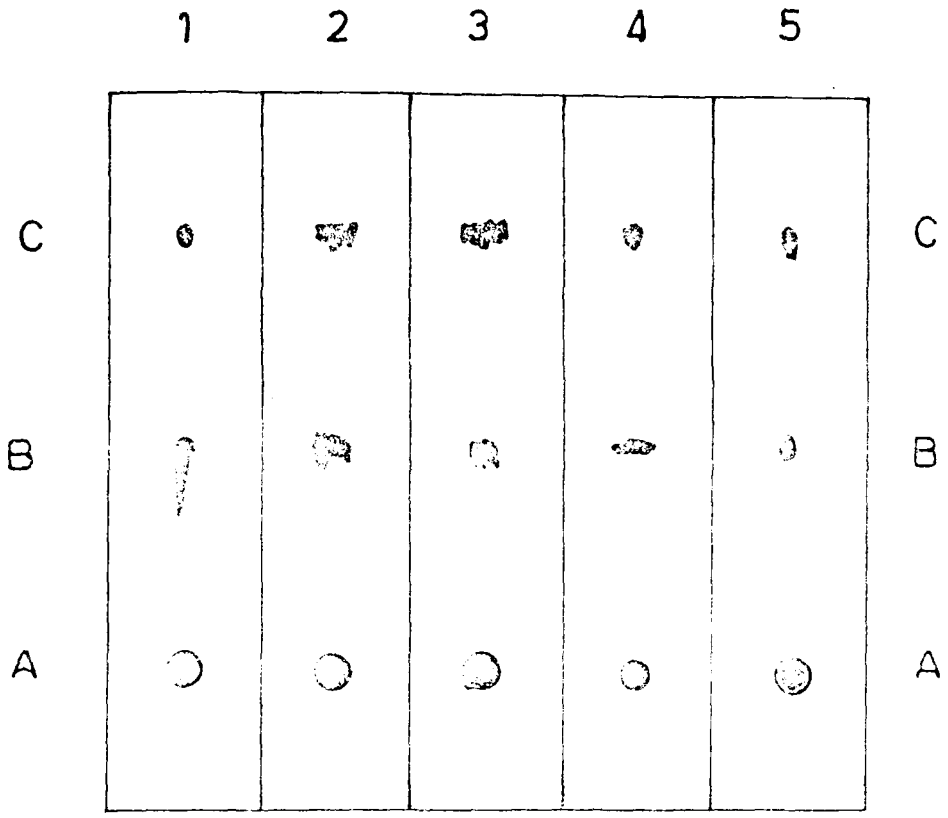
Separation of citric acid (Spot A) from 7) cinnamic acid; from 8) β-naphthaleneacetic acid; from 9) β-naphthoxyacetic acid; from 10) phenoxyacetic acid and from 11) Salicylic acid (Spot B) on calciumsulphate coating, developed in chloroform (5 cm) followed by ethyl acetate (10 cm).



Photoplate No.7

Ternary Separations: Oxalic acid (Spot A) from 1) malic acid (Spot B) and cinnamic acid (Spot C) in chloroform (5 cm) and ethylacetate (10 cm); from 2) indole-3 acetic acid (Spot B) and cinnamic acid (Spot C) in ethyl acetate (5 cm) and carbon tetrachloride; from 3) indole-3-acetic acid (Spot B) and phenoxyacetic acid (Spot C) in ethyl acetate (5 cm) and carbon tetrachloride (10 cm); maleic acid (Spot B) and cinnamic acid (Spot C) in acetone (5 cm) and benzene (10 cm); from 5) maleic acid (Spot B) and phenoxyacetic acid (Spot C) in acetone (5 cm) and benzene (10 cm) on calcium sulphate coated plates.





Photoplate No. 8

Ternary Separations: Tartaric acid (Spot A) from 1) malic acid (Spot B) and cinnamic acid (Spot C) in chloroform (5 cm) and ethyl acetate (10 cm); from 2) indole-3-acetic acid (Spot B) and cinnamic acid (Spot C) in ethylacetate (5 cm) and carbon tetrachloride (10 cm); from 3) indole-3-acetic acid (Spot B) and phenoxy-acetic acid (Spot C) in ethyl acetate (5 cm) and carbon tetrachloride (10 cm); from 4) maleic acid (Spot B) and cinnamic acid (Spot C) in acetone (5 cm) and benzene (10 cm); from 5) maleic acid (Spot B) and phenoxyacetic acid (Spot C) in acetone (5 cm) and benzene (10 cm) on calcium sulphate coated plates.

TABLE 10

BINARY QUANTITATIVE SEPARATIONS OF IAA FROM OA AND TA.

Carboxylic pollutants	N	Separated from	Absorbance at 510 mm $\mu \pm \sigma$	C.V.
50 µg of IAA	6	200 µg of TA	0.214 ± 0.005	2.32
50 µg of IAA	6	400 µg of OA	0.212 ± 0.0098	4.64

Thickness of calcium sulphate layer = 0.75 mm and ec solvent system.

TABLE 11

TERNARY QUANTITATIVE SEPARATIONS OF MA FROM OA AND CIA.

Carboxylic pollutants	N	Separated from	Absorbance at 510 nm $\mu \pm \sigma$	C.V.
50 µg of IAA	6	400 µg of OA and 400 µg of CIA	0.203 ± 0.0081	3.99
50 µg of IAA	6	800 µg of OA and 800 µg of CIA	0.205 ± 0.0137	6.68
50 µg of IAA	6	1200 µg of OA and 1200 µg of CIA	0.191 ± 0.0075	3.95
50 µg of IAA	6	1600 µg of OA and 1600 µg of CIA	0.193 ± 0.0126	6.50

Thickness of calcium sulphate layer = 0.75 mm and ec solvent system.

TABLE 12

TERNARY QUANTITATIVE SEPARATIONS OF IAA FROM TA AND CIA.

Carboxylic pollutants	N	Separated from	Absorbance at 510 mμ $\mu \pm \sigma$	C.V.
50 μg of IAA	6	200 μg of TA and 400 μg of CIA	0.216 ± 0.0081	3.75
50 μg of IAA	6	400 μg of TA and 800 μg of CIA	0.207 ± 0.0081	3.91
50 μg of IAA	6	600 μg of TA and 1200 μg of CIA	0.205 ± 0.0055	2.68
50 μg of IAA	6	800 μg of TA and 1600 μg of CIA	0.215 ± 0.0104	4.83

Thickness of calcium sulphate layer = 0.75 mm and ec solvent system.

DISCUSSION

Results recorded in table 2 show that in P-TLC tailing is observed in forty two cases while compact spots are obtained in four cases only. Seven compounds such as citric acid, p-chlorophenoxyacetic acid, gallic acid, maleic acid, malonic acid, oxalic acid, and tartaric acid stand at the point of application (R_f value is 0.0) while fourteen compounds such as benzoic acid, cinnamic acid, citric acid, p-chlerephenoxyacetic acid, 2,4-dichlorophenoxyacetic acid, gallic acid, indole-3-acetic acid, malic acid, β-naphthaleneacetic acid, β-naphthoxy-acetic acid, phenoxyacetic acid, salicylic acid, trichloroacetic acid and 2,4,5-trichlorophenoxyacetic acid have R_f value 1.0. None of the compounds have R_f value 0.5 on P-TLC. Tables 3, 4 and 5 show that there is a considerable change in the movement of the compound by using S-TLC in place of P-TLC in the same solvents. For example seven compounds have R_f value 0.5 (table 3) on S-TLC while it is not possible on P-TLC. Hence it is obvious that only binary separations can be obtained by P-TLC while ternary separations can be obtained by S-TLC. Table 6 shows that the calculated R_f values are very close to the experimental R_f values by S-TLC. It shows the reproducibility as well as accuracy of S-TLC.

Results summarised in table 7 show that calcium sulphate possesses a good separation potential of herbicidal pollutants

such as cinnamic acid, 2,4-dichlorophenoxyacetic acid, trichloroacetic acid and 2,4,5-trichlorophenoxyacetic acids. Table 8 shows that the binary separations achieved by P-TLC can also be achieved by S-TLC. Table 9 shows that in addition to the binary separations listed in table 8 many ternary separations can also be achieved.

The practical utility of S-TLC is clearly shown by depicting the separations in photoplates 1 to 8. Separations shown in photoplate 1, 3 and 5 can also be obtained by P-TLC while separations shown in photoplates 2, 4, 6, 7 and 8 can not be achieved by P-TLC.

The quantitative separation potential of S-TLC on calcium sulphate in ethylacetate followed by carbon tetrachloride for binary separations of indole-3-acetic acid from oxalic acid and tartaric acid and for ternary separations of indole-3-acetic acid - oxalic acid and tartaric acid - cinnamic acid is shown in tables 10, 11 and 12.

The results discussed above show that S-TLC can be used for the separations which are possible on P-TLC as well as for new separations which are not possible by P-TLC. It is also obvious that calcium sulphate is an excellent TLC material for separating complex mixtures of herbicidal pollutants.

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